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Nerve growth factor improves visual loss in childhood optic gliomas: a randomized, double-blind, phase II clinical trial

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Paediatric optic pathway gliomas are low-grade brain tumours characterized by slow progression and invalidating visual loss. Presently there is no strategy to prevent visual loss in this kind of tumour. This study evaluated the effects of nerve growth factor administration in protecting visual function in patients with optic pathway glioma-related visual impairment. A prospective randomized double-blind phase II clinical trial was conducted in 18 optic pathway glioma patients, aged from 2 to 23 years, with stable disease and severe visual loss. Ten patients were randomly assigned to receive a single 10-day course of 0.5 mg murine nerve growth factor as eye drops, while eight patients received placebo. All patients were evaluated before and after treatment, testing visual acuity, visual field, visual-evoked potentials, optic coherence tomography, electroretinographic photopic negative response, and magnetic resonance imaging. Post-treatment evaluations were repeated at 15, 30, 90, and 180 days Brain magnetic resonance imaging was performed at baseline and at 180 days. Treatment with nerve growth factor led to statistically significant improvements in objective electrophysiological parameters (electroretinographic photopic negative response amplitude at 180 days and visual-evoked potentials at 30 days), which were not observed in placebo-treated patients. Furthermore, in patients in whom visual fields could still be measured, visual field worsening was only observed in placebo-treated cases, while three of four nerve growth factor-treated subjects showed significant visual field enlargement. This corresponded to improved visually guided behaviour, as reported by the patients and/or the caregivers. There was no evidence of side effects related to nerve growth factor treatment. Nerve growth factor eye drop administration appears a safe, easy and effective strategy for the treatment of visual loss associated with optic pathway gliomas.

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Abbreviations: OPG = optic pathway glioma; PhNR = photopic negative response; RGC = retinal ganglion cell; RNFL = retinal nerve fibre layer; VEP = visual-evoked potential

Introduction

Paediatric optic pathway gliomas (OPG) are usually low-grade brain tumours with a slow progression (Binning *et al.*, 2007). Their major cause of morbidity is the progressive visual loss associated with infiltration and compression of the optic pathways by tumour cells. Retinal ganglion cells (RGCs) are thought to be a major target of disease-induced visual damage in OPG (Hegedus *et al.*, 2009; Kim *et al.*, 2010; Gu *et al.*, 2014). The retinal nerve fibre layer (RNFL), the most proximal region of the afferent visual pathway comprised by RGC axons, has been considered a structural marker of visual integrity in patients with OPG (Gu *et al.*, 2014). Reduced RNFL thickness due to RGC axonal loss has actually been associated with clinically evident visual loss in children with OPGs.

At present, no specific therapy is available for OPG-induced visual impairment (Kalin-Hajdu *et al.*, 2014). Nerve growth factor (NGF) was the first discovered neurotrophin. Originally known for its role in the development and survival of sympathetic neurons, NGF has proved effective in promoting neural recovery after inflammatory, ischaemic, and toxic injuries in a large number of experimental and clinical models (Verge *et al.*, 1992; Chiaretti *et al.*, 2008).

In the optic pathways, NGF receptors are found in RGCs, whose axons form the first part of the optic pathways (Carmignoto *et al.*, 1991; Caleo *et al.*, 2000), as well as in the visual cortex (Rossi *et al.*, 2002; Tropea *et al.*, 2002). In both regions, NGF administration can ameliorate visual function, as shown by preclinical studies. Intraocular NGF administration has been shown to delay or prevent RGC loss and the concomitant visual loss induced by optic nerve transection and ocular ischaemia in experimental animal models (Carmignoto *et al.*, 1991; Porciatti and Ventura, 2012). When directly applied in the visual cortex, NGF can boost glutamate release and improve visual responses to impoverished visual inputs (Sala *et al.*, 1998; Pizzorusso *et al.*, 1999).

Testing the effectiveness of NGF in human visual pathologies has been hindered by the search for non-invasive administration strategies to bypass the blood–retina barrier. At present NGF administration, in the form of eye drops, appears a promising way to circumvent this difficulty. Recent experimental animal studies showed that NGF applied on the conjunctiva can reach the retina and the optic pathway (Lambiase *et al.*, 2005), as well as the cerebral cortex (Capsoni *et al.*, 2009), exerting biological activities in these regions. Clinical studies have also shown the safety and effectiveness of NGF eye drops in patients with corneal ulcers (Lambiase *et al.*, 2007), as well as in patients with severe glaucoma (Lambiase *et al.*, 2010).

In a previous pilot open-label, longitudinal study involving five paediatric patients suffering from severe visual impairment associated with OPG, no adverse effect was found on tumour growth, but rather a promising transient increment in visual-evoked potential (VEP) amplitude in all treated patients (Falsini *et al.*, 2011). Furthermore, in an adult patient with a bilateral pre-chiasmatic OPG, a dramatic improvement in visual acuity and visual field was observed following repeated short-term courses of NGF eye drop treatment (Chiaretti *et al.*, 2011). As promising as these results have been, the possibility of a placebo effect and examiner bias was inherent in this kind of open-label pilot study, as was the risk of other potential sources of bias, such as improvement due to repeat testing and inter-session variability.

For these reasons, the present randomized, double-blind, placebo controlled clinical study was designed to evaluate the efficacy of NGF eye-drop administration in protecting visual function in patients with OPG-associated visual loss.

Materials and methods

Eligibility

Patients with OPG-induced visual impairment, with or without neurofibromatosis type 1 (NF1), admitted to the Division of Paediatric Oncology at the 'Agostino Gemelli' Hospital in Rome (Italy) were enrolled from September 2012 to September 2013 (Table 1). A histopathological diagnosis was not required in the setting of characteristic MRI and clinical features. Additional eligibility criteria included: age $\leqslant$25 years; clinically documented visual impairment; no concomitant ophthalmological disorders that could affect electrophysiological assessment; no radiotherapy or chemotherapy within 12 months prior to entry; stable disease at two brain MRI controls, performed at least 6 months apart. The study was conducted in accordance with Good Clinical Practice, the Declaration of Helsinki and local regulations. Written informed consent was signed by patients $\geqslant$18 years and by parents or their legal guardians for patients younger than 18 years. Evidence of assent was obtained from the children. The protocol was approved by the Ethics Committee of the Sacred Heart Catholic University in Rome (Italy). This is a registered clinical trial (EudraCT n. 2011-003030-14).

Study design

A two-arm, placebo-controlled, randomized clinical trial was performed. Patients were randomly (1:1) assigned, in a double-blind manner (both clinicians and patients blind to

Table 1 Demographic and clinical data of enrolled patients

Gender	
Male (*n*)	11
Female (*n*)	7
NF1, *n* (%)	13 (72.2)
Age at enrolment	
Median (years)	11.4
Range (years)	2–23
Time from diagnosis to study entry	
Median (years)	7.5
Range (years)	1.7–19.6
Time from last treatment to study entry	
Median (years)	4.59
Range (years)	0.3–13.3
Type of treatment	
Biopsy, *n* (%)	8 (44.4)
Chemotherapy, *n* (%)	14 (77.7)
Radiotherapy, *n* (%)	1 (5.5)

the treatment), to receive NGF or placebo, i.e. an identical solution not containing NGF. A total of 0.5 mg NGF diluted in 2.5 ml saline solution was administered to the conjunctiva of both eyes three times a day, for 10 consecutive days. In each treatment we administered one drop of the diluted solution per eye [1 drop = 41.6 µl containing 8.33 µg of NGF (0.05 mg of NGF/daily)]. This amount is considered sufficient to reach and stimulate NGF receptors, mainly tyrosine receptor kinase A (TrkA), in most cerebral cholinergic areas of the brain and optic pathways, as previously reported (Eriksdotter Jönhagen *et al.*, 1998; Chiaretti *et al.* 2008). The placebo was administered according to the same schedule. At baseline, treated and placebo cohorts consisted of 10 and eight patients, respectively. After drop-outs for lack of compliance, they comprised nine and eight patients, respectively.

Safety assessment

Patients were assessed by medical history and physical examination at baseline, and 15, 30, 90 and 180 days post-treatment (Supplementary Table 1). The safety analysis was based on the evaluation of adverse events, symptoms related to study medication instillation (i.e. burning/stinging/itching, stickiness, foreign body sensation and blurred vision), ocular signs at slit lamp examination, and treatment tolerability as judged by the investigator and patient or parent/guardian. For preverbal children, unusual discomfort upon instillation was assessed by parent/guardian. In all patients, if an exacerbated reaction was noted by the parent/guardian upon instillation of the study medication to the child, the symptoms of burning/stinging, stickiness, foreign body sensation and blurred vision were recorded. Moreover, potential systemic complications related to NGF administration were monitored, including systemic pain, weight loss, and allergic reactions. MRI (brain and orbits) were performed at baseline and 180 days after NGF treatment, using standard imaging parameters and gadolinium enhancement. Initial and follow-up scans were reviewed by a neuroradiologist to evaluate tumour extent and location, and record any changes after treatment. Progression of the OPG mass, as reflected by a volumetric increase of >25% from baseline in brain MRI, was regarded as a potential adverse outcome event. Unacceptable toxicity or disease progression led to discontinuation of the study medication.

Neuroradiological assessment

Optic gliomas (namely pilocytic astrocytomas) represent a heterogeneous group of tumours because their extent and their magnetic resonance signal intensity are extremely variable. This kind of tumour may be limited to one or two intra-orbital optic nerves, may localize mainly to the chiasm, may form a giant hypothalamic mass, or may involve diffusely all optic pathways, from the optic nerves to the occipital cortex. The signal intensity and degree of contrast enhancement of optic gliomas are heterogeneous, and signal changes may occur spontaneously, even without any treatment. An expert neuroradiologist assessed pre- and post-treatment MRIs of all 18 OPG patients in the study, to recognize changes in size and signal behaviour in the tumour tissue. Moreover, in patients examined in our neuroradiological facilities (12/18), we obtained a more reliable and reproducible measurement of these tumours, before and after NGF treatment; thus we decided to select the T_2-weighted and post-contrast T_1-weighted axial slices, where the tumour is largest, and measured the largest diameters and the perpendicular ones. In tumours limited to one optic nerve, we measured the longest axis of the involved nerve and the transverse diameter. In tumours centred in the optic chiasm, we measured the perpendicular diameters. In tumours with prevalent hypothalamic growth, we measured the largest diameters of the bulk mass and the perpendicular one. The measures were obtained using both T_2 and post-contrast T_1-weighted axial slices in order to avoid over- or under-estimation of the heterogeneous enhancing tumours.

Visual outcome measures

All patients were tested at baseline and at 15, 30, 90 and 180 days post-treatment. Visual function tests included physiological subjective and electrophysiological objective measures. The former comprised the early treatment diabetic retinopathy study (ETDRS), visual acuity test, and Goldman visual field perimetry. The latter included Ganzfeld electroretinograms and flicker-induced VEPs. In addition, retina monitoring by optical coherence tomography was performed at each time point.

The following main outcome variables were considered: (i) best corrected EDTRS visual acuity (BCVA); (ii) Goldman perimeter visual field size (V/4 e isopter); (iii) amplitude and latency of the electroretinographic photopic negative response (PhNR)—a valuable tool for monitoring longitudinal change in RGC function in humans (Abed *et al.*, 2015); (iv) amplitude and latency of the first and second harmonic components of the flicker VEP recording—a tool for monitoring visual cortical function, which has proved reliable in monitoring OPG progression (Falsini *et al.*, 2008); and (v) the thickness of RNFL obtained from optical coherence tomography screening, a measure whose alteration has been shown to correlate with visual loss in a cross-sectional study of OPG in children (Avery *et al.*, 2011). All parameters were evaluated in terms

of their changes from baseline. Given the very low vision conditions of some patients, visual acuities and visual fields were expected to be less reliable than electrophysiological evaluations (see below). Consequently, in designing the study, it was decided that physiological measures of visual function would be included as secondary outcome measures, while electrophysiological (VEP and electroretinogram) and anatomical (optical coherence tomography RNFL) measures were the main outcomes in the evaluation of potential drug efficacy.

Sample size considerations and statistical analysis

Preliminary evaluations suggested that ~30 patients aged <25 years, with OPG, would be followed by the clinical centre. Based on a previous pilot study in five OPG patients (Falsini *et al.*, 2011), this number was estimated to give a power of 80% at an alpha = 0.05 for detecting an average change difference between the NGF and placebo of at least 30% in VEP amplitude. Clinical and electrophysiological data were initially log-transformed to limit skewness. However, the statistical analysis produced similar results when using the original scale. Ordinal variables (i.e. presence of adverse events such as periocular pain, changes in pupillary responses, changes in visual field extension) were analysed by non-parametric tests. In the preliminary analyses, multilevel regression techniques were applied to take into account the potential correlation between the eyes of each subject. However, visual measures between the two eyes of each patient were substantially uncorrelated (data not shown), as one would expect from the asymmetry of OPG. Thus, each eye measure was considered independently. Visual acuity, visual field size, PhNR and VEP amplitude were initially evaluated separately by multilevel regression models for repeated measures, where group (NGF-treated versus placebo-treated patients) was the between-subjects factor and time (i.e. the different recording sessions including baseline and post-treatment visits) the within-subjects factor. Analyses were performed using raw data and changes from baseline. Finally a seemingly unrelated equation regression (Sureg) model was performed where changes from baseline at each time of all the six electrophysiological experimental outcomes (i.e. amplitude and latency of PhNR, VEP first and second harmonic, RNFL, and optical coherence tomography measures) were included as dependent variables, and the independent variables were the arm group and the baseline values of each parameter. This analysis is a generalization of a linear regression model that consists of several regression equations, each having its own dependent variable and potentially different sets of exogenous explanatory variables. Each equation is a valid linear regression in its own right and can be estimated separately, but the error terms are assumed to be correlated across the equations. In all the analyses, a P-value < 0.05 was considered statistically significant.

Results

Baseline clinical and demographic characteristics, tumour location and tumour size of patients are shown in Table 2. At this time there was no significant difference in age, gender, tumour location, size, and visual function parameters between NGF and placebo-treated patients.

Safety

Both NGF and placebo treatments were well tolerated in all patients, with no severe ocular adverse events reported. Ocular adverse events considered by the investigator as related to the study drug were reported in two patients (20%) treated with NGF eye drops (Supplementary Table 2). These included periocular burning lasting <10 min after drug application. All patients of the NGF group reported that they had experienced phosphenes, often several times in one day. These were characterized variously as moving horizontal and/or vertical lines, circles or semi-circular shapes, crescent moons, lightning bolts, swirling waves, and flashes covering the entire visual field. However, all treatment-related ocular adverse events were mild. Neither corneal inflammation, nor active inflammation of the anterior chamber was noted in any patient on slit lamp examination. Overall, patient/guardian-rated and investigator-rated tolerability was good. MRI control examinations performed at baseline and 6 months after the end of treatment did not show any disease progression in either NGF- or placebo-treated patients (Supplementary Fig. 1). In six patients, tumour size measurements could not be reliably performed due to non-homogenous morphology of the lesions and/or ill-defined margins (Table 2). Optic coherence tomography did not show retinal alteration over the study period.

Efficacy evaluation: electrophysiological results

Examples of electroretinogram and VEP curves in a NGF and a placebo-treated patient are shown in Fig. 1 at baseline and at one time during the treatment. Individual curves representing the time course of the amplitude variation from baseline in the PhNR (top) and the VEP first harmonic (bottom) in individual eyes of NGF- (left) and placebo- (right) treated patients are shown in Fig. 2. A considerable degree of variability is evidenced from case to case, but only placebo-treated patients presented major worsening, while the greatest improvements from baseline were observed in NGF-treated patients. This qualitative difference is particularly evident for the VEPs. These same data are summarized as means and standard deviations (SD) in Fig. 3. Multivariate analysis with Sureg regression including all six electrophysiological experimental outcomes (amplitude and latency of PhNR, VEP first and second harmonic performed on eyes with no complete loss of visual function, RNFL and optical coherence tomography measures) showed significant effects of the treatment on the PhNR and the VEP first harmonic amplitude. In particular, this model showed statistically significant mean differences between NGF-treated and placebo group of PhNR

Table 2 Clinical characteristic, treatment, and tumour location of patients

Patient	Group	Gender	Age at enrolment (years)	Tumour location	Biopsy	Previous treatment		Pretreatment size (mm)	Post-treatment size (mm)	Time from diagnosis to study entry (years)	Time from last treatment to study entry (years)
						RT	CT				
1	T	M	8	Optic nerves, chiasm	N	N	VCR, CBDA, CDDP	N.A.*	N.A.*	5.2	0.3
2	T	F	7.5	chiasm, tracts/ radiations	Y	N	N	26 × 17	25 × 17	3.1	3.1
3	T	M	16.1	Optic nerves, chiasm, tracts/radiations	N	N	N	N.A.*	N.A.*	14.7	N.A.
4	P	F	20.8	Optic nerves, chiasm	N	N	VCR, CBDA	23 × 13	24 × 12	18	13.2
5	T	F	16.35	Right optic nerve, optic-diencephalic site	N	N	VCR, CBDA	17 × 10	14 × 12	8	13.1
6	T	M	6.3	Right optic nerve, chiasm	N	Y	VCR, CBDA, CDDP, CYC	N.A.*	N.A.*	5	0.64
7	P	M	23.1	Left optic nerve and chiasm	Y	N	VCR, CBDA, CVDP	N.A.*	N.A.*	19.6	13.3
8	T	M	11	Optic nerves, chiasm, diencephalon, tracts/radiations	Y	N	VCR, CBDA	22 × 12	22 × 11	7.6	5.8
9	P	F	15.8	Retrochiasmatic area	Y	N	N	N.A.*	N.A.*	1.7	1.7
10	P	F	7.8	Optic nerves, chiasm, diencephalon, tracts/radiations	N	N	VCR, CBDA	24 × 13	23 × 12	5.6	4.1
11	T	M	11.8	Optic-diencephalic site	Y	N	VCR, CBDA	43 × 15	43 × 14	7.5	6.4
12	P	F	21.6	Retrochiasmatic area	Y	N	VCR, CBDA, CCNU, Procarbazine, CDDP, VP-16	N.A.*	N.A.*	15.2	9.8
13	T	M	13.9	Optic nerves,chiasm, tracts/radiations	N	N	N	27 × 12	25 × 12	11.9	N.A.
14	T	M	9.7	Optic nerves, chiasm, diencephalon,tracts/ radiations, retro-chiasmatic area	N	N	VCR, CBDA	34 × 22	35 × 23	7	5.9
15	T	M	12.8	Optic nerves, chiasm, tracts/radiations, retrochiasmatic area	N	N	VCR, CBDA	29 × 14	28 × 15	11	1.8
16	P	M	9.1	Left nerve optic	N		VCR, CBDA	21 × 14	22 × 14	6.5	5
17	P	F	10.7	Optic nerves, chiasm, diencephalon, tracts/radiations, retrochiasmatic area	Y	N	VCR, CBDA, CDDP, VBL	16 × 11	15 × 11	9.9	1.1
18	P	M	2.2	Optic-diencephalic site	Y	N	VCR, CBDA, VP-16	46 × 32	47 × 31	2	0.57

T = treatment; P = placebo; M = male; F = female; Y = yes; N = no; RT = radiotherapy; CT = chemotherapy; VCR = vincristine; CBDA = carboplatin; CDDP = cisplatin; CYC = cyclophosphamide; VP-16 = etoposide; VBL = vinblastine. N.A.* = not applicable, because in these patients only qualitative assessment were obtained. None of the patients showed significant tumour changes at the end of follow-up compared to baseline.

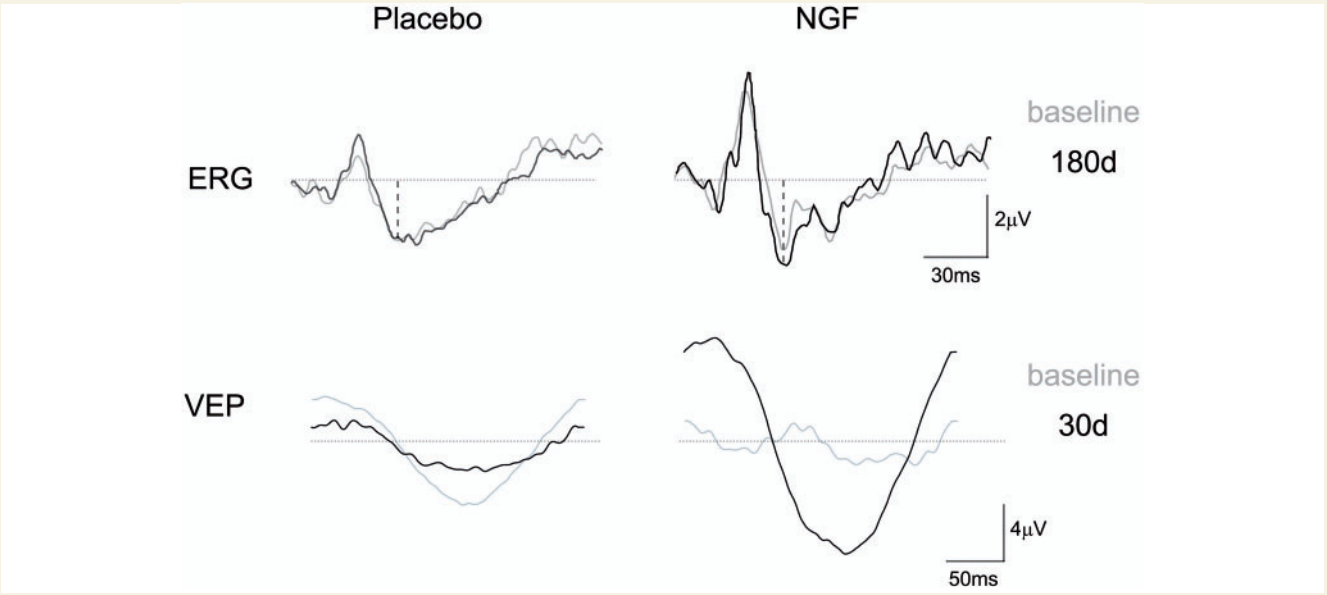

Figure 1 Examples of electroretinogram and VEP curves recorded from a NGF- and a placebo-treated subjects. Grey lines represent baseline recording. Black lines represent recordings at the specified times. The electroretinogram PhNR component is the negative deflexion from baseline indicated by the dotted lines in the traces. d = days.

amplitude at 180 days ($P < 0.01$), and of PhNR latency at 15 days ($P < 0.01$) and at 180 days ($P = 0.02$) and of VEP first harmonic amplitude at 30 days ($P < 0.01$). Statistically significant differences between the two study groups were not observed in any of the other objective visual outcome measures (Table 3).

Efficacy evaluation: clinical results

Reliable visual field measures were obtained for 18 eyes, including 10 NGF- and eight placebo-treated eyes, from six NGF- and five placebo-treated patients, respectively. As established in the study design, visual field modifications were evaluated in double-blind manner by two experts, acting independently of one another. Visual fields were scored as improved, stable, or worsened compared to baseline. Expert scoring was coincident. Their results are shown in Fig. 4A, where, for the sake of simplicity, only the percentage of cases with a visual field modification are reported. Visual field improvement was more frequent in the NGF- than in placebo-treated eyes (five versus one eyes, three versus one patients, respectively). Furthermore, visual field worsening was only observed among placebo-treated eyes. Examples of the variation of the visual field limits observed with two different targets in NGF- and placebo-treated eyes are shown in Fig. 4B, which illustrates a significant enlargement compared to baseline in the NGF-treated case. This enlargement was never observed in placebo-treated patients. Visual acuity did not show any significant change over the trial in either NGF- or placebo-treated eyes.

Efficacy evaluation: subjective patient/caregiver reports

Patients spent the most part of the study in their familiar surroundings, but could always get in touch with the senior oncologist to report observations and difficulties. Four of the NGF-treated patients/caregivers reported improvement of visually guided behaviour in the patient (e.g. ability to walk through doorways without assistance). These included the three NGF patients with improved visual fields. No subjective improvement was reported for any placebo-treated patient.

Discussion

This randomized, double-blind, placebo controlled, clinical study was designed to evaluate the safety and efficacy of conjunctively applied NGF in children with OPG-associated visual impairment. We found that NGF treatment led to significant improvements in objective electrophysiological parameters (PhNR amplitude and VEP), which were not observed in placebo-treated patients. Furthermore, among patients retaining measurable visual fields (50% of the cases), visual field restriction was only observed in placebo-treated cases, while one-third of the NGF-treated cases showed significant visual field enlargement. This corresponded to improved visually guided behaviour, as reported by the patient and/or the caregivers (who, due to the study design, were blind to the treatment nature). There was no evidence of adverse effects, or serious ocular or

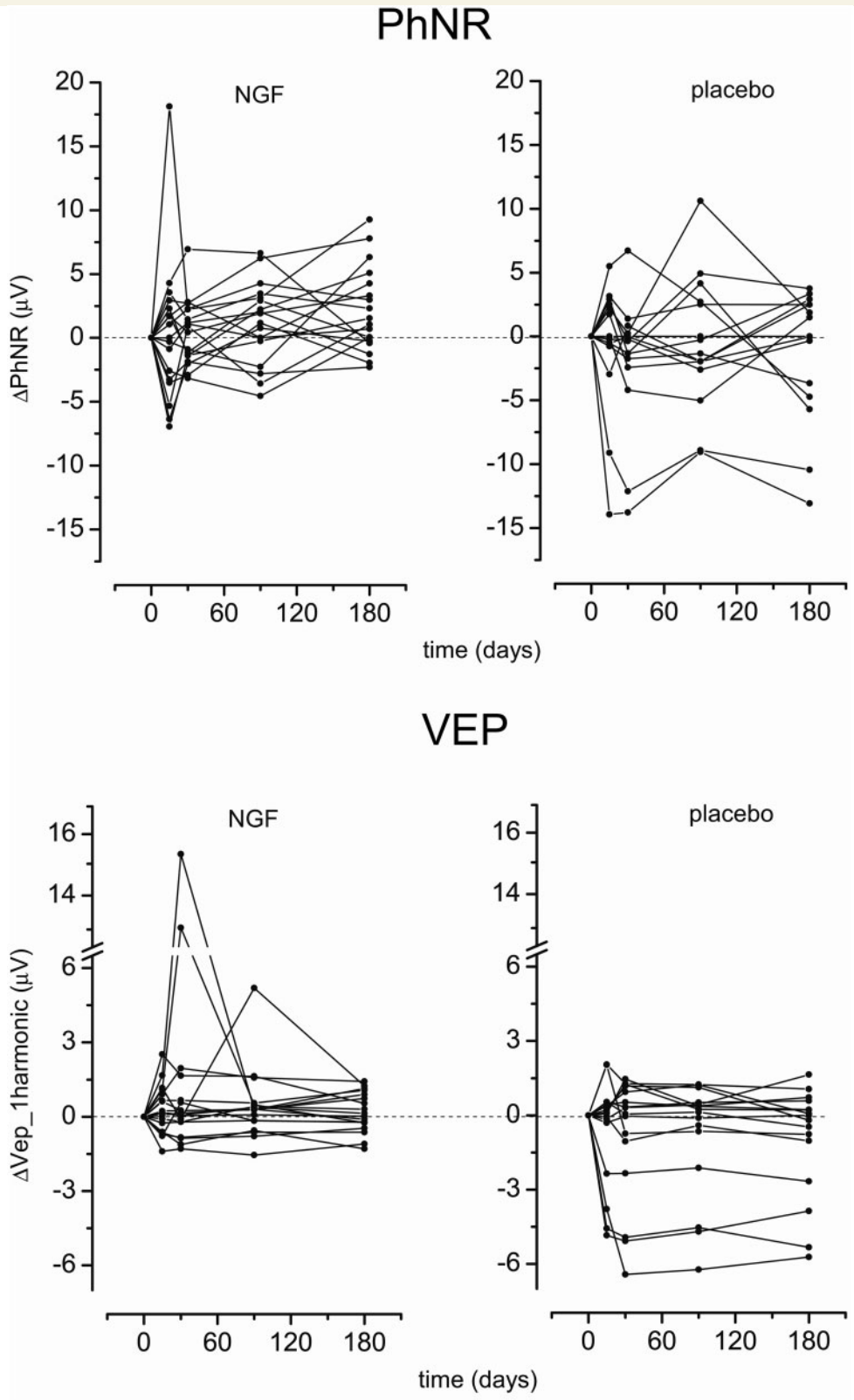

Figure 2 Individual time courses of PhNR and VEP first harmonic amplitude variation from baseline in NGF- and placebo-treated patients. Variations from baseline are represented as absolute values. Each curve represents a single eye. *Top*: PhNR amplitude; *Bottom*: VEP first harmonic.

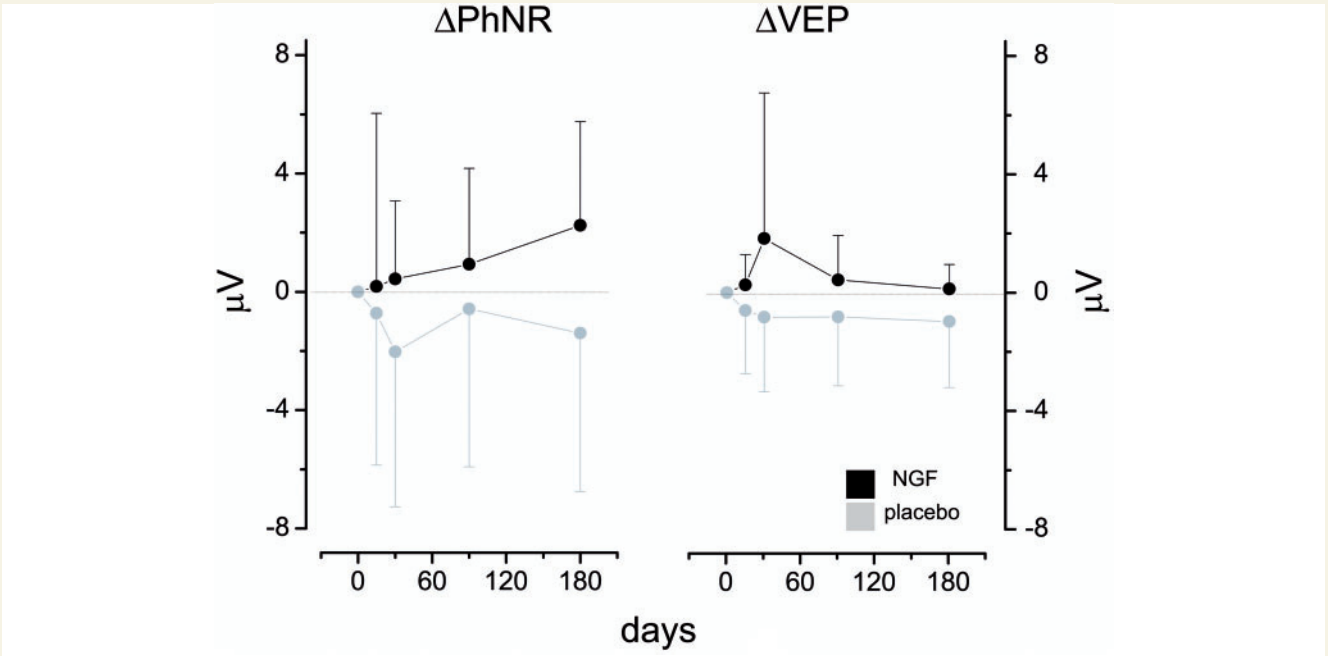

Figure 3 Mean amplitude variation in PhNR and VEP first harmonic over time in NGF- and placebo-treated patients. Data are represented as means and SD.

Table 3 Adjusted estimates of mean differences between NGF and placebo arm for several optical parameters obtained from seemingly unrelated equations regression model; adjustment is made for the optical parameters at baseline

	Days since treatment start	Estimate	95% CI	*P*-value
PHNR amplitude	15	1.17	(−0.99;3.34)	0.29
	30	1.57	(−0.60;3.73)	0.16
	90	2.05	(−0.11;4.22)	0.06
	180	**3.11**	**(0.79;5.43)**	**0.01**
PHNR latency	15	**1.47**	**(0.56;2.37)**	**<0.01**
	30	0.55	(−0.36;1.45)	0.24
	90	0.59	(−0.31;1.50)	0.20
	180	**1.16**	**(0.19;2.13)**	**0.02**
VEP amplitude I	15	0.18	(−2.42;2.78)	0.89
	30	**3.72**	**(1.12;6.32)**	**0.01**
	90	0.05	(−2.55;2.64)	0.97
	180	−0.20	(−2.98;2.58)	0.89
VEP amplitude II	15	−0.37	(−0.90;0.16)	0.17
	30	0.07	(−0.46;0.59)	0.80
	90	0.23	(−0.29;0.76)	0.39
	180	0.10	(−0.47;0.66)	0.74
RNFL	15	−6.48	(−16.81;3.85)	0.22
	30	−1.82	(−12.14;8.51)	0.73
	90	0.27	(−10.06;10.60)	0.96
	180	−5.40	(16.43;5.63)	0.34
OCT	15	6.34	(−18.79;31.49)	0.62
	30	−21.03	(−46.16;4.11)	0.10
	90	9.31	(−15.83;34.44)	0.47
	180	8.15	(−18.60;34.91)	0.55

Note: estimated parameters for optical values at baseline are not reported in the table. Values highlighted in bold indicate a statistical significance.
OCT = optical coherence tomography.

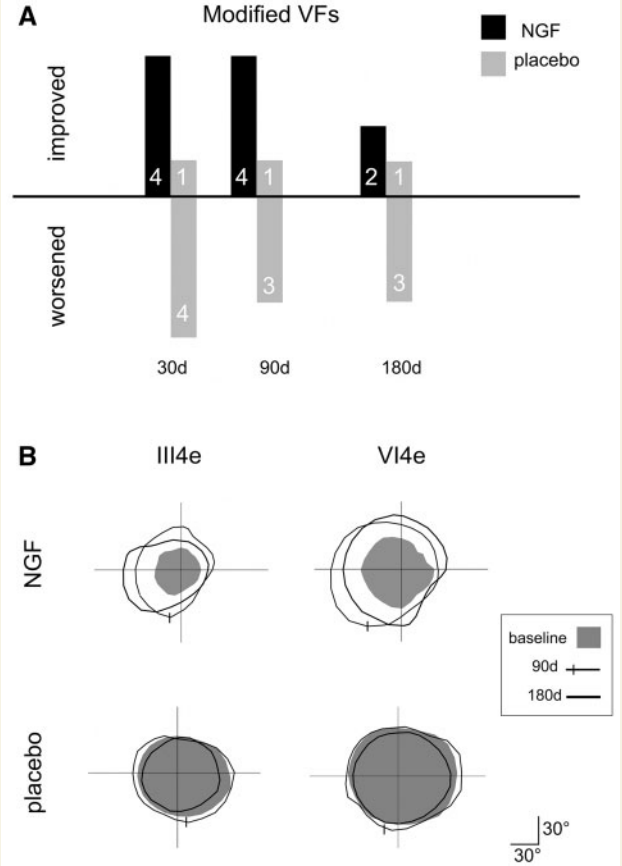

Figure 4 Visual field variations in NGF- and placebo-treated patients. (**A**) Number of improved and worsened visual fields (VFs) in NGF- and placebo-treated patients at various times after treatment. The actual numbers of visual fields is reported on each bar. Visual field assessment was performed qualitatively in double-blind by two independent observers. (**B**) Examples of visual field variations from baseline during the trial in a NGF- and a placebo-treated subject. Traces are obtained using the III4e (*left*) and V4e (*right*) targets. d = days.

systemic safety issues related to NGF treatment. MRI displayed stable tumour size.

One first important issue to consider is the lack of adverse effects of the treatment. NGF eye-drops have already been tested in a number of clinical eye conditions, including corneal ulcers and glaucoma. None of the patients included in such studies experienced significant adverse effects either topically or systemically (Lambiase *et al.*, 2007, 2010). Similarly, no adverse effect was observed in OPG patients enrolled in our previous pilot open-label studies with NGF eye drops (Falsini *et al.*, 2011). These studies represented our base reference in the trial design. In line with these studies, no major visual adverse effect was observed in the present study. A critical issue in the study design was the risk of adverse effects of the treatment on tumour progression. A low risk expectation was based on two levels of observation. Low-grade astrocytomas are characterized by markedly reduced (~75% less) NGF and TrkA expression, and upregulation of the pro-apoptotic p75 NGF receptor (Chiaretti *et al.*, 2004). It is postulated that this is related to the slow growth of these tumours, and to the potential differentiating role of NGF on tumour cells. In line with this view, astrocytoma and low grade glioma cells can undergo differentiation when exposed to NGF *in vitro* (Kraft *et al.*, 2001), and preclinical experiments show inhibition of glioma cell proliferation following NGF *in vivo* administration (Verge *et al.*, 2002). For these reasons we considered it unlikely that a limited acute dose of NGF administered as eye drops would interfere with OPG growth. In line with this view, none of the six patients treated in our previous pilot studies showed tumour growth either in the 6-month period of the study (Chiaretti *et al.*, 2011; Falsini *et al.*, 2011), or in the 2 years that have now elapsed since their treatment. This expectation has been confirmed in the present study where no tumour growth was observed 6 months after the trial short-term NGF treatment, as assessed by expert neuroradiological review of brain MRI scans taken at baseline and at 6 months from the trial. The main expectations from the trial was the proof-of-principle of a non-invasive therapeutic strategy to counteract the visual decline that is a constant and still unmet feature of OPGs. Our two previous open-labelled studies had already raised hopes that NGF treatment could provide such a tool, by showing improved VEP in treated patients. The present double-blind study provides solid grounds for such an expectation, showing that, although with considerable variability from patient to patient, NGF treatment led to improved objective and subjective visual parameters, which are not found in placebo-treated patients. While the possibility of a placebo effect and examiner bias was inherent in our previous open-label pilot studies, this can likely be excluded in the present double-blind design, as neither the examiner nor the patient or caregivers were aware of whether the subject was receiving treatment or placebo. The double-blind design also enables other potential sources of bias to be excluded, such as improvement due to repeat testing and intersession variability, as these would have affected NGF- and placebo-treated patients alike. Significantly improved objective and subjective visual outcomes in treated patients, together with the lack of similar effects in placebo-treated cases, suggests biological activity of NGF treatment. NGF receptors are found on the RGCs—whose axons form the pre-geniculate part of the optic pathway, as well as in the visual cortex. In animal models, intra-ocular NGF has proved able to preserve RGC function following ischaemic or traumatic insults (Verge *et al.*, 1992; Siliprandi *et al.*, 1993; Lambiase *et al.*, 2009; Sivilia *et al.*, 2009; Bai *et al.*, 2010). Similarly, when directly applied in the visual cortex, NGF can boost excitatory inputs, improve visual responses (Pizzorusso *et al.*, 1999; Galuske *et al.*, 2000), and promote visual cortical plasticity (Maffei *et al.*, 1992). In the present study, we found that a short (10 day) period of treatment resulted in a long-term (3–6 months) stabilization/improvement of visual function. The period of treatment and the

cumulative dose were chosen on the basis of our previous pilot studies (Chiaretti *et al.*, 2011; Falsini *et al.*, 2011), indicating that this treatment regimen was safe, and showing promising functional results, suggesting efficacy. Our intent was to test the hypothesis that this short treatment would be able to show a significant effect when compared to placebo. The long-term effect on visual function is consistent with a prolonged change in visual neurons following NGF exposure. Different biological activities have been proposed to explain the mechanisms of action of NGF on the visual system. In animal models, NGF binding to TrkA upregulates BCL2 protein levels, which protects RGC from apoptosis by preventing caspase activation (Coassin *et al.*, 2008). Following a single NGF administration, the transcriptional activity activated by the NGF pathway induces a pro-survival change in BAX/BCL2 balance and C-Jun expression in RGCs, with neuroprotective effects that may last several months (Sivilia *et al.*, 2009). Based on these studies, we speculate that neuroprotection of RGCs by NGF may represent a main mechanism active in eliciting the reported prolonged effects of NGF in children with optic gliomas. We cannot exclude, however, that the effects of NGF may relate not only to a protective activity against neural apoptosis. NGF is known to act at different levels to promote neuronal recovery following ischaemic, inflammatory and traumatic injuries: through neosynaptogenetic mechanism, by promoting axonal regeneration (Shimohama *et al.*, 1993), by regulating miRNA expression (Shi, 2015), and by directly affecting induction of other growth factors, such as BDNF, whose neuroprotective effects on RGCs has been reported (Chen *et al.*, 2001; Frost, 2001). Moreover, it has also been demonstrated that ocular administration of NGF could elicit an NGF-mediated neuroprotective effect in several brain nuclei (Calza *et al.*, 2001; Tirassa, 2011), including brain regions and cells not strictly related to the optic pathway. Thus, an improvement of visual cortex physiology elicited by ocular NGF treatment cannot be excluded.

Together with tumour progression, visual decline in OPG is the most important reason that paediatric neuro-oncologists take into account for introducing more aggressive therapy, including chemotherapy and/or radiotherapy (Fisher *et al.*, 2013). These treatments can have devastating effects on some patients, and their efficacy in preserving visual function is variable (Moreno *et al.*, 2010; Fisher *et al.*, 2013), particularly in the long term (Shofty *et al.*, 2011). A potential alternative strategy for dealing with visual deficits in OPG, as shown here with NGF treatment, could allow a separate management of visual deficits and tumour progression, potentially reducing the need or the frequency of more aggressive treatments in this kind of tumour.

In conclusion, the significant improvement of electrophysiological and visual subjective measures in treated eyes, taken with the absence of significant vision loss in treated eyes, and the lack of significant improvement in placebo-treated eyes, is a promising finding that merits further investigation. As this study included a small number of patients we cannot exclude that some potential positive effects of NGF, compared to placebo, have been missed for some parameters. However, we should take into account that this study was a phase 2 trial and its main objective was to identify the possible therapeutic efficacy of this new treatment. A phase 3 trial with a larger number of subjects and including more clinical centres should be considered to confirm these encouraging results. These results suggest that NGF eye drops could provide a potentially safe strategy to ameliorate visual function in OPGs. This is the first report of a non-aggressive strategy with beneficial effects on visual function in childhood OPG.

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Supplementary material

Supplementary material is available at *Brain* online.

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